Observations of Microzooplankton in the Vicinity of Whale Shark *Rhincodon typus* Aggregation Sites in Oslob, Cebu and Pintuyan, S. Leyte, Philippines

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ABSTRACT

The whale shark is the world's largest fish yet 60-90% of its diet is made up of the smallest prey, the zooplankton. The species is listed as endangered under the International Union for Conservation of Nature Red List of Threatened species. The biology and ecology of this species are largely unknown. Although parts of their movements have been mapped out, their food, which plays a major role in their travels and habitat use, is hardly studied. We therefor analysed zooplankton community composition along whale shark aggregation sites in Pintuyan, Southern Leyte, where sharks occur seasonally from November to June; and Oslob, Cebu, where they are hand-fed and found daily year- round. Water samples were taken in stations in each site during November 2014, January and February, 2015. In both sites with mostly microzooplankton of the Order Tintinnida was the most abundant group with a density of 2,000 indiv/m^3 and 3,000 indiv/m^3 in Pintuyan and Oslob, respectively. Other zooplankton observed were from classes Appendicularia, Ascidia, Bivalvia, Crustacea, Gastropoda, Ophiuroidae, Polychaeta, and Sarcodina. In total, the densities in both sites were not significantly different. Oslob registered a density value of 5,000 indiv/m^3 and SB had 4,000 indiv/m^3. In contrast, Pintuyan had a higher diversity value (2.75) than Oslob (2.36). Our results highlight that although whale sharks were not actively feeding near the sampling sites, the waters near their feeding sites are both abundant and rich in zooplankton diversity. Further studies to quantify and understand their target prey are needed.

KEYWORDS

Microzooplankton, Whale shark, aggregation sites, Cebu

INTRODUCTION

Zooplankton are tiny animals found in all aquatic environments. These heterotrophic organisms function in linking energy from the primary producers to higher organisms. These are part of the diet of various types of organisms stretching from mesozooplankton (Calbet, 2008), juvenile fish (Nielsen and Munk, 1998), and whale sharks (Richardson, 2008).

Whale sharks (*Rhincodon typus* Smith 1828) are highly mobile species with their movements primarily associated with the presence of food, mostly zooplankton such as copepods, Motta et al., 2010; sergestid shrimp, fish spawn, Robinson et al., 2013; Rohner et al., 2015), and others. Whale sharks are mostly spotted throughout tropical and warm temperate waters (Colman, 1997), oceanic, and coastal regions (Rowat & Brooks, 2012) including the Philippines (Araujo et al., 2014). Whale sharks can be individually identified by their unique spot pattern and thus data can be minimally-invasively collected to understand population dynamics, movements and residency (Marshall & Pierce, 2012).

Whale sharks, like basking sharks *Cetorhinus maximus* Gunnerus 1765 and megamouth sharks *Megachasma pelagios* Taylor, Compagno & Struhsaker 1983, are filter feeders. They filter large amounts of water, through their specially adapted gills, catching plankton, fish, squid and other prey items (Heyman *et al.*, 2001; Ketchum *et al.*, 2013; Rohner et al., 2013; Motta et al., 2010). Unlike basking sharks and megamouth sharks, whale sharks can use suction feeding where they target dense concentrations of planktonic and small nektonic preys (Heyman *et al.*, 2001; Hacohen-Domené *et al.*, 2006).

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Zooplankton are the largest fraction in their diet (Rohner et al., 2013, Hernández-Nava and Álvarez-Borrego, 2013), including but not limited to sergestid shrimp (Rohner et al., 2015) and copepods (Motta et al., 2010). Instead of being species-selective, whale sharks target high biomass of zooplankton patches (Rohner et al., 2015).

Zooplankton, which are about 60 or 70-90 % of whale sharks' diet (Silas and Rajagopalan, 1963, Rohner et al., 2013), are believed to be one of the environmental cues of whale shark migration. Whale sharks can support successful tourism industries at sites where they predictably aggregate. For example, whale shark tourism at the Maldives was valued between U$7.6 and U$9.4 million (Cagua et al., 2014), and in Ningaloo Reef, Australia, at AU$12.5 million (Huveneers et al., 2017). In the Philippines, whale sharks currently support three described tourism operations: Donsol, Sorsogon, Pintuyan, Southern Leyte, and Oslob, Cebu (Quiros, 2007; Araujo et al., 2014; 2017 respectively). In Pintuyan, Sogod Bay (SB), whale sharks occur between November and June (Araujo et al., 2017); and at Oslob, Cebu (OC), they are hand-fed year-round (Thomson et al., 2017). There is a need for zooplankton assessments for sustainable management and practices to be implemented. This will provide important information that will help explain whale shark movements in Philippines and, in turn, help in the conservation efforts of the world's largest fish.

Relationship between Zooplankton and Migratory Fish

Zooplankton feeders such as macrocrustacea and fish migrate and follow their prey (Iwasa, 1982). In Bangladesh, plankton composition, diversity, and abundance were evaluated with respect to its relationship to hilsa (*Tenualosa ilisha*), a diadromous fish species that migrate only through the Ganges□ Meghna river system route (Ahsan *et al.*, 2012). Of which, 39 taxa (67.24 %) of zooplankton were identified on the site which restricts the migration of the fish upstream and induces spontaneous spawning. A study in Yucatan Peninsula, Mexico, they calculated that whale sharks ingest 1467 and 2763 g of plankton per hour (Motta et al., 2010).

Whale Shark Distribution

Whale sharks are found in tropical and warm temperate waters, with predictable aggregations primarily associated with food (Rowat & Brooks, 2012). Whale sharks are widely distributed worldwide from tropical and warm temperate seas (Colman, 1997) to oceanic and coastal regions (Gunn *et al.*, 1999) around the equator between about 30°N and 35°S (Compagno, 1984). Because they are migratory, they transfer from one region to another depending on the amount of food and change in abiotic factors. In the western Pacific, they prefer areas where the surface temperature is 21-25 °C with cold water of 17 °C or less upwelling into it and salinity of 34.0-34.5 ppt (Compagno, 1984). Whale sharks have been spotted in Atlantic Ocean (Afonso, 2014), Western Australia (Colman, 1997; Taylor, 2007), Gulf of California in Mexico (Hacohen-Domené *et al.*, 2006; Nelson and Eckert, 2007; Hernández-Nava and Álvarez-Borrego, 2013), and Madagascar (Compagno, 1984). Locally named "butanding" whale sharks are regular visitors in the waters of the tropical country, the Philippines (Snow, 2013).

Whale Shark Diet and Feeding Behavior

The whale shark, is characterized externally by having a broad and flattened head, large and terminal mouth, and unique "checkerboard" pattern of light spots and stripes on their bodies (Compagno, 1984). Function of the distinctive pattern on their bodies can either be adaptation to radiation shielding (Colman, 1997), camouflage in pelagic environments (Wilson and Martin, 2003), or species recognition processes (Myrberg, 1991). The spot pattern can also be harnessed for capture-mark-recapture population studies (see Marshall & Pierce, 2012).

Whale sharks, one of the three large pelagic species, are filter feeders that suck a large amount of water primarily feeding on a variety of planktonic and small nektonic organisms (Heyman *et al.*, 2001). Zooplankton including calanoid copepods and sergestid shrimps (Craven, 2012), mysids, chaetognaths, and decapod larvae are reported as their food (Silas and Rajagopalan, 1963; Colman, 1997). Typically, copepods dominate their usual diet (Compagno, 1984; Lavaniegos *et al.*, 2012). Whale sharks also prey on small schooling fish such as sardines, anchovies, and mackerels and larger prey aquatic animals such as small tunas, albacores, and squids (Colman, 1997). Stomach analyses revealed that accident ingestion of animal associates such as suckerfish (Karbhari and Josekutty, 1986; Prater, 1941) can also happen when feeding or during capture. Moreover, phytoplankton and seaweeds were observed from the stomach analysis conducted by Karbhari and Josekutty (1986). Large masses of algae were also observed by Wright (1870) and McCann (1954) which suggests an omnivorous diet.

Zooplankton and Whale Shark Studies

Large marine animals, such as whale sharks, must consume large amounts of food to obtain

sufficient energy. However, these large planktivores generally inhabit warm oligotrophic waters (Compagno, 1984; Colman, 1997), where their food are sparsely distributed (Rohner *et al*., 2015). As a result, whale sharks move to areas with high-biomass prey patches, wherein most aggregations appear to target specific prey items (Rohner et al., 2015). In Yucatan Peninsula, Mexico, whale sharks target dense concentrations of sergestids, calanoid copepods, chaetognaths, and fish larvae (Motta *et al*., 2010). Crustaceans are also reported to dominate their feeding grounds such as euphausiids *Pseudeuphausia latifrons* and portuniid megalopa along Ningaloo Reef (Taylor, 2007), copepods *Acartia* (Lavaniegos *et al*., 2012; Hernández-Nava and Álvarez-Borrego, 2013), *Undinula*, and *Corycaeus* in Gulf of California (Hacohen-Domené *et al*., 2006), and sergestids *Lucifer hanseni* in Tanzania (Rohner *et al*., 2015). Moreover, whale sharks in Belize Barrier Reef feed on freshly released spawn of cubera snappers and dog snappers (Heyman *et al*., 2001). In Bahia de Los Angeles, coast of Gulf of California, seasonal variability of cladocerans *Pseudevadne tergestina* and *Penilia avirostris* was observed, wherein the former species occurred whole year round and the latter in summer and autumn only (Lavaniegos *et al*., 2012). Studies of Nelson and Eckert (2007) and Ketchum *et al*. (2013) revealed that whale sharks change their feeding behaviours based on zooplankton composition.

METHODOLOGY

Sampling was done monthly in Sogod Bay, Southern Leyte and Oslob, Cebu in three sampling stations on each site. This was done in November 2014, January and February, 2015 at 8:00 am to 10:00 am, synchronized at whale shark feeding times in both sites (Quiros *et al*., 2007; Araujo *et al*., 2014).

Study Site

Sogod Bay, Southern Leyte

Barangay Son-ok is a village located in the municipality of Pintuyan, on the island of Panaon, province of Southern Leyte, Philippines. The west side of the island faces Sogod Bay, which is a part of Bohol Sea (Calumpong et al., 1994). It is an important fishing ground in the province where large supply of fish- such as tunas, herrings, anchovies, mackerels, and flying fish- and mega-fauna- such as pilot whales, dolphins, and melon-headed whales- are found (Calumpong et al., 1994). Whale sharks have been seasonally sighted, between November to July, on the eastern side of Sogod Bay due to the current patterns created by the channel at the town of Liloan (Araujo *et al*., 2017). However, after supertyphoon Haiyan, locally called Yolanda, hit the area last November 8, 2013, the whale shark spotted in the site decreased (Araujo *et al*., 2017). Studies on whale shark here have focused on the population of animals that visit these waters, and how tourism can affect them (Araujo et al., 2017)

Oslob, Cebu

Oslob, a municipality in the southern end of Cebu, Philippines, encompasses 21 barangays including Tan-awan, where whale sharks are handfed daily, year round since late 2011 (Araujo et al., 2014). The practice began when fishermen led the whale sharks, which they considered a nuisance, away from their fishing areas using uyap, a small species of shrimp (Araujo et al., 2014). Then some enterprising fishermen started luring the whale sharks toward the shore and charging tourists to see the big fish. Now, provisioning takes place between 6:00 am and 12:30 pm in a 65,457 m^2 semi-circular interaction area (Araujo et al., 2014). An average of 12.7 whale sharks are present daily in the interaction area (Araujo et al., 2014). Previous studies conducted in Oslob focused only on the population, behavior, and ecology of whale sharks (Craven, 2012; Araujo et al., 2014). There is no account of zooplankton studies in this site. This study aims to measure the physico-chemical parameters in Sogod Bay and Oslob; determine the composition and abundance of zooplankton by major taxonomic groups in both sites; and to compare the abundance and different zooplankton species found therein.

Sampling

The sampling stations' locations in Sogod Bay were based on preliminary work by Muncada (2014). Stations 1 and 2 were positioned near the tip of Pintuyan (N 9°54'27.0", E 125°15'51.42") and near Benit Port, San Ricardo (N 9°54'53.69", E 125°17'32.7"). However, station 3 was relocated from deep water to near Pintuyan town proper (N 9°57'25.92", E 125°14'26.16"). See figure 1.A. The new established station was the location with most whale shark spotting before super typhoon Haiyan hit the area (Cordova, pers. comm.). Establishment of the stations was authorized by the Local Government Units of Pintuyan and San Ricardo.

In Tan-awan, Oslob, whale shark interaction is a daily tourist activity. Sampling stations in this area were established with permission from the Local Government Unit of Oslob. Station 1 was situated outside the whale shark interaction area (N 9°27'43.34", E 123°22'52.38"). Stations 2 and 3 were

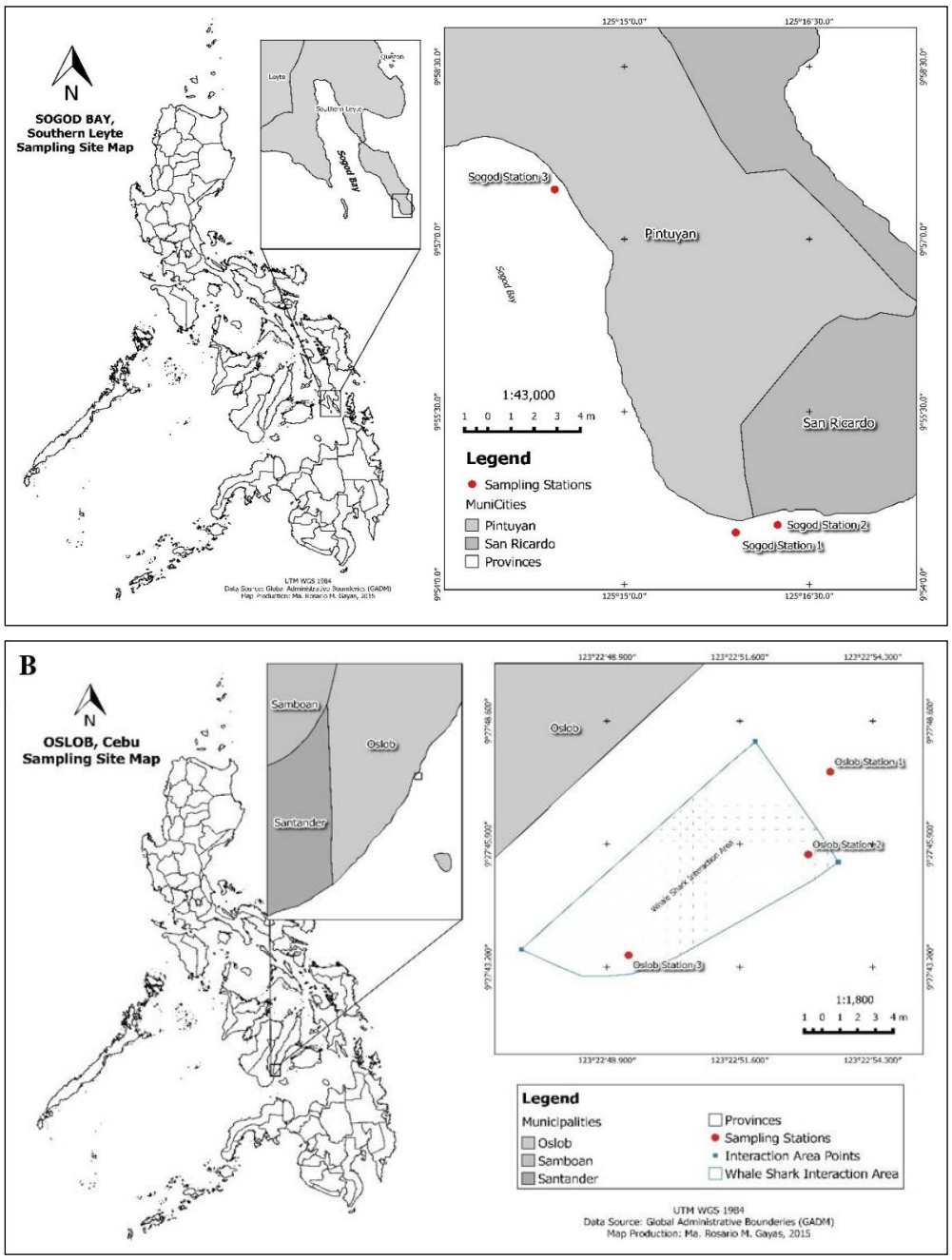

Figure 1. Sampling stations (red dots) in A. Sogod Bay, Southern Leyte from November 2014 to February 2015. The black star refers to station 3 of the previous study of Muncada (2014) and in B. Oslob, Cebu

positioned inside the interaction area (N 9°27'45.0", E 123°22'52.5" and N 9°27'42.3", E 123°22'49.98"). See figure 1.B. The coordinates were taken by a handheld Garmin Oregon 550 GPS receiver unit and were plotted in QGIS.

Field Sampling

Physico-chemical Analysis

Physico-chemical parameters of the water including temperature, pH, salinity, were assessed using a centigrade field thermometer, EUTECH pH meter, and a handheld Atago refractometer, respectively. Water current was evaluated with the use of a fabricated holey sock drogue. Depth and turbidity were estimated with the use of a Secchi disc. The following formula determined the turbidity in NTU (Nephelometric Turbidity Unit) of the water (Kearns, 2008): $\log(T) = -1.4249 \log(d) + 3.2935$ where T is turbidity and d is distance (cm) of disappearance of the Secchi disc.

For nutrient (phosphate and nitrate) analyses, 500 ml water sample was filtered through Whatman GFC glass-fiber filters (1.2 µm pore size). The filtrate was collected by an acid-washed bottle, sealed in a parafilm and wrapped in a poltethylene bag. After freezing, the samples were sent to Chemical Oceanography Laboratory of the Marine Science Institute of University of the Philippines-Diliman for analyses.

Collection of Samples

Plankton samples were collected using 1 m long conical plankton net with 30 cm mouth diameter and 20 µm mesh size. The plankton net was lowered 1 m below the surface and was towed vertically. The collected water sample was dispensed to 50 ml pre-labelled bottle and was fixed by adding 0.5 ml Lugol's solution. The procedure was done twice in every station. Afterwards, the samples were brought to the laboratory for zooplankton identification.

For quantitative analysis, a 2.2 L capacity WILDCO alpha sampler was lowered at 1 m below the water surface. Water collected was dispensed to 1 L pre-labelled container and was preserved by adding 10 ml Lugol's solution. This method was done twice in every station. Afterwards, the samples were brought to the laboratory to initiate settlement of the zooplankton.

Plankton Settling Method

Prior the quantitative analysis, the preserved 1 L water samples were left undisturbed for at least 24 hours to settle zooplankton. After settling, capillary tube was used to siphon the upper 800 ml of the water sample and the remaining was transferred to 250 ml graduated cylinder. This was left undisturbed for at least another 24 hours. After which, about 150 ml of supernatant was siphoned using the same process in the first decantation. The remaining approximately 50 ml concentrated sample was transferred to amber bottles for analysis.

Zooplankton Identification

One milliliter water sample was placed in a Sedgewick rafter and was viewed under 100x magnification of a Yuan XSP 91-05 light compound microscope; Photomicrographs, taken by a Samsung Note 3 N-9005. At least three trials of aliquot or until there are no new species found was done. The manuals Coastal Plankton Photo Guide for European Seas (Larink and Westheide, 2006) and Illustrations of Marine Plankton of Japan (Yamaji, 1966) were used for identification.

Zooplankton Density Determination

The 50 ml concentrated water samples were used for density determination. After shaking to even out the zooplankton, 1 ml aliquot was placed in a Sedgewick rafter counting chamber, which was viewed under a light compound microscope. Three trials, each trial counting at least 300 cells, were done for each sample. The density was obtained using the formula:

$$\text{Density} = \frac{\text{no. of cells}}{\text{ml}} \times \frac{\text{ml of concentrated sample}}{\text{1L}}$$

Data Analyses

Zooplankton richness, diversity, and evenness were determined using the following formulae (Krebs, 1989):

Menhinick index **(Richness)** $D = \frac{S}{\sqrt{N}}$

Shannon-Weiner index **(Diversity)** $H' = -\sum_{i=1}^{S} p_i \ln(p_i)$

Pielou index **(Evenness)** $J' = \frac{H'}{\ln(S)}$

where: H'= Shannon-Weiner index

p_i= fraction of the entire population made up of family *i*

S= number of families encountered

N= total number of individuals

T-tests were applied to compare the density and abundance between Sogod Bay and Oslob, Cebu.

RESULTS

Physico-chemical Parameters

pH values between the two sites were observed to be at normal range with an avergae of 9.1 for Sogod Bay (SB) and 9.3 for Oslob Cebu (CB), respectively. In both sites, January displayed the highest value of 9.3 (SB) and 9.6 (OC) compared with November and February. In terms of temperature, SB displayed a mean value of 26.8°C. Temperatures measured in January and February were almost the same with values 26.7°C and 26.5°C, while November had a higher value of 27.1°C, which is probably because it was the only month with sunny weather throughout the sampling period. Temperatures were comparatively the same in OC with a mean value of 27.2°C. Salinity values were relatively the same throughout the sampling period, which ranged from 34.1-34.8 and 33.7-33.9 ppt in SB and OC, respectively. See Table 1.

Table 1. Mean values of physico-chemical parameters in A. Sogod Bay, Southern Leyte and B. Oslob, Cebu from November 2014 to February 2015

A. Sogod Bay	pH Level	Temperature (°C)	Salinity (ppt)	Current (ms^{-1})	Turbidity (NTU)
November	8.96 ± 0.03	27.13 ± 2.14	34.11 ± 0.77	0.07 ± 0.04	0.11 ± 0.03
January	9.25 ± 0.03	26.66 ± 0.35	34.77 ± 0.60	0.05 ± 0.04	0.10 ± 0.02
February	9.03 ± 0.02	26.45 ± 0.48	34.44 ± 0.51	0.07 ± 0.03	0.10 ± 0.01
Average	**9.08**	**26.75**	**34.44**	**0.06**	**0.10**

B.Oslob, Cebu	pH Level	Temperature (°C)	Salinity (ppt)	Current (ms^{-1})	Turbidity (NTU)
November	9.36 ± 0.37	27.11 ± 0.42	33.89 ± 0.51	0.04 ± 0.02	0.17 ± 0.05
January	9.61 ± 0.07	27.44 ± 0.51	33.67 ± 0.34	0.04 ± 0.02	0.12 ± 0.02
February	8.98 ± 0.02	27.00 ± 0.50	33.89 ± 0.51	0.02 ± 0.01	0.13 ± 0.01
Average	**9.32**	**27.18**	**33.82**	**0.03**	**0.14**

In every month, water currents changed depending on the weather and time when the parameter was obtained. In SB, both November and February showed average water current of 0.07 ms^{-1}, while January had the weakest water current of 0.05 ± 0.04 ms^{-1}. Moreover, in every month, water current was observed to be strongest at station 2 with 0.10 ± 0.01 ms^{-1}, while stations 1 and 3 displayed lower values with 0.05 $ms^{-1}$and 0.04 ms^{-1}, respectively. This can be attributed to the location of station 2, which is situated near Benit Port, San Ricardo, facing the Surigao Strait. On the other hand, in OC, November and January had the same water current of 0.04 ms^{-1}. Station 1 had the strongest water current with 0.05 ± 0.02 ms^{-1}. Turbidity values in November, with a mean value of 0.11 NTU in SB and 0.17 NTU in OC, were higher than that of January and February. In SB, station 1 was recorded to be the deepest with an average depth of 23.50 ± 3.50 m, with low turbidity value of 0.08 NTU (transparency: 12.06 m). In OC, station 2, recorded to be the deepest station, had an average depth of 12.25 ± 2.25 m and a turbidity value of 0.11 NTU (transparency: 9.64 m). Refer to table 2.

Phosphate and nitrate concentrations in SB and OC were analysed in January and February only. See figure 2. Phosphate concentrations observed between the two sites were within the acceptable range with an average of 0.09 µM for SB and 0.08 µM for OC, respectively. In SB, February displayed 0.11 µM phosphate, a higher concentration than that of January with 0.07 µM only. In contrast, in OC, January had a higher concentration of 0.10 µM compared to February with 0.06 µM. The highest phosphate concentration was recorded in station 2 of SB in February with 0.11 µM, while the lowest was observed in January in the same location with 0.02 µM. Nitrate concentrations range from 0.9-4.27 µM in SB and 0.71-3.43 µM in OC. In both sites, January had higher values, with 3.54 µM in SB and 3.08 µM in OC, compared with February. The acceptable ranges were set by the Association of Southeast Asia Nations (ASEAN, 2008), wherein the values should not exceed 0.48 µM for phosphates and 4.28 µM for nitrates.

Qualitative Analysis

The zooplankton observed during the analyses were identified up to family level. In some cases, individuals were categorized into a group or up to class level only since they were morphologically indiscernible from one another under ordinary light microscope. This includes groups of copepod nauplii,

Table 2. Mean values of depth, current, and turbidity per station from November 2014 to February 2015 sampling in Sogod Bay (SB) and Oslob, Cebu (OC)

	Depth (m)		Current (ms^{-1})		Turbidity (NTU)	
	SB	OC	SB	OC	SB	OC
Station 1	23.50 ± 3.50	10.00 ± 1.00	0.05 ± 0.03	0.05 ± 0.02	0.08 ± 0.02	0.14 ± 0.01
Station 2	9.75 ± 1.25	12.25 ± 2.25	0.10 ± 0.01	0.03 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
Station 3	10.25 ± 2.75	8.75 ± 0.75	0.04 ± 0.02	0.03 ± 0.01	0.12 ± 0.02	0.16 ± 0.06

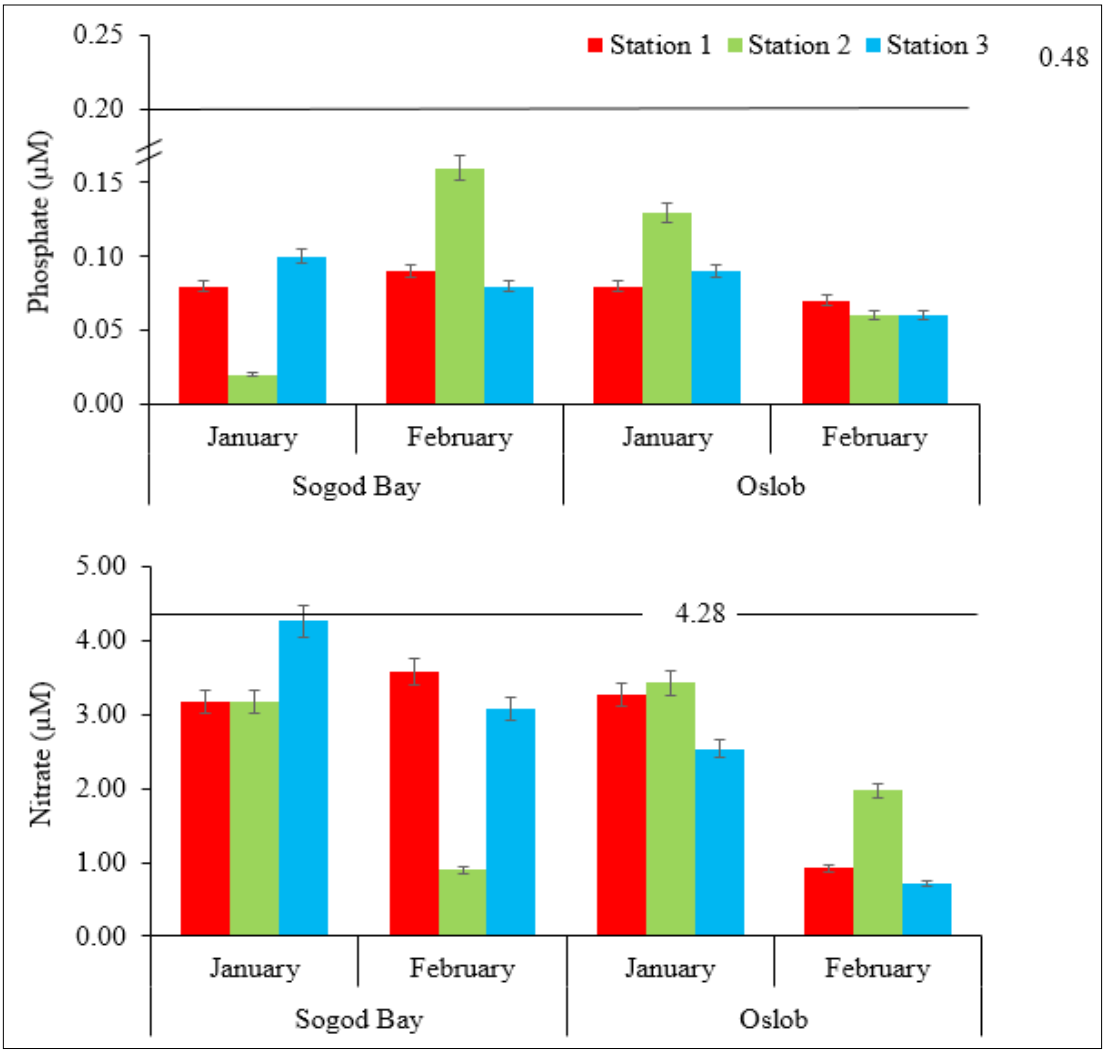

Figure 2. Summary of phosphate and nitrate mean values between Sogod Bay and Oslob during January and February 2015. Lines are accepted concentration levels set by the ASEAN Marine Water Quality Criteria. (ASEANt, 2008)

teleost eggs, euphausiid nauplii, polychaete larvae and class Ophiuroidea. A total of 33 zooplankton groups, which include 28 families from seven phyla, were observed from plankton net samples in both sites. Twenty-eight zooplankton groups were observed in SB. The same zooplankton groups, with five more families, were observed in OC. The additional zooplankton were families Aegisthedae, Centropagidae, Tintinnidae, Sagittidae, and Luciferidae. Figure 4 shows some zooplankton groups from different phyla observed in both sites.

In both sites, phylum Arthropoda contributed the most number of families (eight families in SB and 11 in OC), wherein most come from subclass Copepoda. There were six famililes under subclass Copepoda in SB which include families Calanidae, Corycaeidae, Oithonidae, Oncaeidae, Paracalanidae, and Tachidiidae. The same families were observed in OC, added with families Aegisthedae and Centropagidae. The zooplankton observed were dominated next by the protozoans. Tintinnids from Class Ciliata contributed five families in SB and six in OC. Other identified zooplankton were from phyla Annelida, Chordata, Chaetognatha (in OC only), Mollusca, and Echinodermata among others. The complete list is recorded in Table 3.

Quantitative Analysis

From the 33 zooplankton groups seen from plankton net collection, only 30 of them were observed from the water sampler: 25 groups were mutually observed between the two sites, two families (Acanthochiasmidae and Sabellariidae) were exclusive to SB, and three families (Centropagidae, Tachidiidae, Tintinnidae) were observed in OC only. Figure 3 compares the mean zooplankton abundance per class between the two sites during the three-month sampling period. Class Ciliata was the most abundant class in both sites comprising 51.35 % in SB and 74.52 % in OC of the total population. From this class, tintinnids from subclass Spirotricha got the highest count in both sites with an average density of 2,000 indiv/m^3 in SB and 3,000 indiv/m^3 in OC. The second most abundant were the polychates in Sogod Bay. The least abundant zooplankton group in both sites came from Class Ascidea, represented in the study by Family Cionidae, the tunicates only, which comprised 1.44 % in SB and 0.18 % in OC, respectively.

Some families were only observed in certain stations or months. Euphausiid nauplii and families Centropagidae, Sabellariidae, and Acanthochiasmidae were observed in January only; while families Limacinidae and Podonidae in OC where observed in November only. Moreover, family Balanidae was only detected in station 3 in SB and across stations 1 and 2 in OC.

The highest density in SB was recorded in station 3 in January with 8,000 indiv/m^3, while the lowest was observed in station 1 in November with 3,000

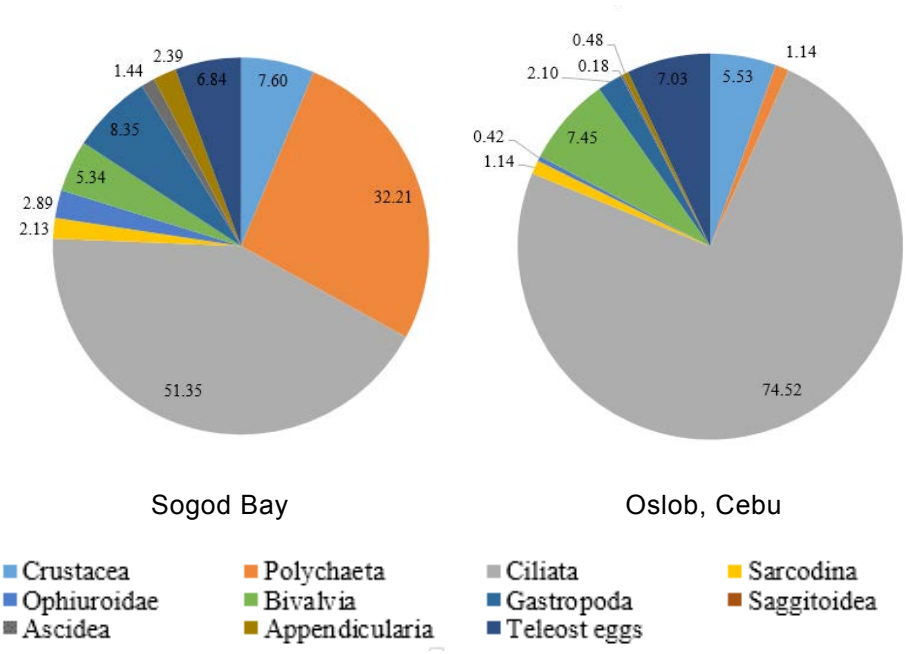

Figure 3. Mean zooplankton abundance per class in Sogod Bay and Oslob, Cebu from November 2014 to February 2015.

Table 3. Presence of Zooplankton Groups in Sogod Bay, Southern Leyte and Oslob, Cebu. (o =absent, x =present)

Zooplankton Groups	Sogod Bay		Oslob 2014-2015
	2013-2014 (Muncada, 2014)	2014-2015	
Phylum Arthropoda, Class Crustacea			
Subclass Copepoda, Order Calanoida			
Family Calanidae	x	x	x
Family Centropagidae (calanoid copepods)	o	o	x
Family Paracalanidae	x	x	x
Order Cyclopoida, Family Oithonidae	x	x	x
Order Harpacticoida			
Family Aegisthedae (calanoid copepods)	o	o	x
Family Ectinosomatidae	x	o	o
Family Tachidiidae	o	x	x
Order Poecilostomada			
Family Corycaeidae	x	x	x
Family Oncaeidae	x	x	x
Copepod nauplii	x	x	x
Subclass Phyllopoda, Order Diplostraca			
Family Podonidae	o	x	x
Subclass Maxillopoda, Order Sessilia			
Family Balanidae	x	x	x
Subclass Eumalacostraca			
Euphausiid nauplii	o	x	x
Order Decapoda, Family Luciferidae	o	o	x
Phylum Annelida, Class Polychaeta			
Unidentified polychaetes	o	x	x
Family Polygordiidae	o	x	x
Order Spionidae, Family Spionidae	o	x	x
Order Sabellida, Family Sabellariidae	x	x	x
Order Phyllodocida, Family Phyllodocidae	x	o	o
Phylum Echinodermata, Class Ophiuroidea	x	x	x
Phylum Mollusca			
Class Gastropoda, Order Thecosomata			
Family Cavolinidae	o	x	x
Family Limacinidae	x	x	x
Order Littorinimorpha, Family Atlantidae	x	o	o
Class Bivalvia, Order Veneroida			
Family Veneridae	x	x	x
Phylum Protozoa			
Class Ciliata, Order Tintinnida			
Family Codonellidae	x	x	x
Family Codonellopsidae	o	x	x
Family Cyttarocylidae	o	x	x
Family Rhabdonellidae	x	x	x
Family Tintinnidae	x	o	x
Family Tintinnididae	o	x	x
Class Sarcodina, Order Radiolaria			
Family Acanthochiasmidae	o	x	x
Order Foraminifera, Family Globigerinidae	o	x	x
Phylum Chaetognatha, Class Sagittoidea			
Order Aphragmophora, Family Sagittidae	o	o	x
Phylum Chordata			
Teleost eggs	x	x	x
Class Ascidea, Order Phlebobranchia			
Family Cionidae (sea squirts)	o	o	x
Class Appendicularia, Order Copelata			
Family Oikopleuridae	x	x	x
Phylum Phoronida, Family Phoronidae	x	o	o

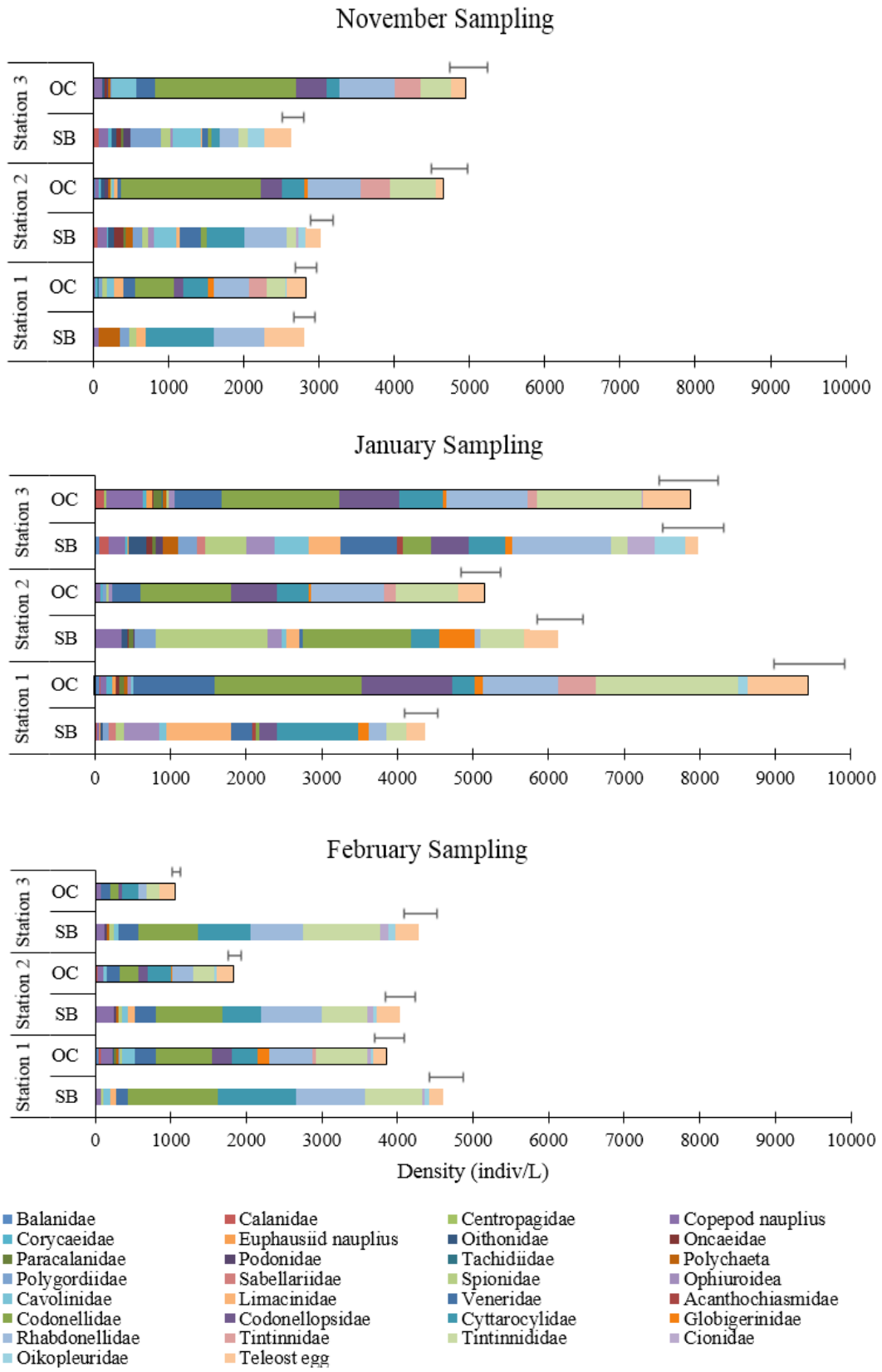

Figure 4. Comparison of zooplankton density in three stations of Sogod Bay (SB) and Oslob, Cebu (OC) in the months of November 2014, January and February 2015

Table 4. Diversity indices in Sogod Bay and Oslob, Cebu from November 2014 to February 2015

	Menhinick Richness index (D)	Shannon-Weiner Diversity index (H')	Pielou Evenness index (J')
Sogod Bay	2.87	2.75	0.84
Oslob, Cebu	2.91	2.36	0.71

indiv/m^3. See figure 4. On the other hand, the highest density in OC was recorded in station 2 in January with 9,000 indiv/m^3. Lesser zooplankton composition was observed during February, which got the lowest density (1,000 indiv/m^3) in station 3 in OC.

Accounting all the data gathered in every stations, January displayed the highest density (6,000 indiv/m^3 in SB and 7,000 indiv/m^3 in OC) throughout the whole sampling period. In contrast, November in SB, with 3,000 indiv/m^3, and February in OC, with 2,000 indiv/m^3, possessed the lowest density. Figure 5 shows the mean zooplankton abundance of SB and OC in different sampling months. In comparison, OC had more abundant zooplankton than SB. OC got an average density of 5,000 indiv/m^3 for the whole sampling period, while SB got a lower value of 4,000 indiv/m^3.

Data Analyses

A higher Menhinick richness was calculated in OC, with a value of 2.91, than SB. This can be attributed to the greater number of zooplankton groups present in OC. SB got only 28 zooplankton groups which was five fewer groups than OC. In contrast, a higher Pielou evenness and Shannon-Weiner diversity were calculated in SB, with a value of 0.84 and 2.75, respectively. This demonstrates that zooplankton were more distributed equitably in SB despite the fact that more zooplankton groups were found in OC. Moreover, the evenness and diversity values in OC were only 0.71 and 2.36, respectively. See Table 4. There was no significant difference on the density and zooplankton abundance between SB and OC.

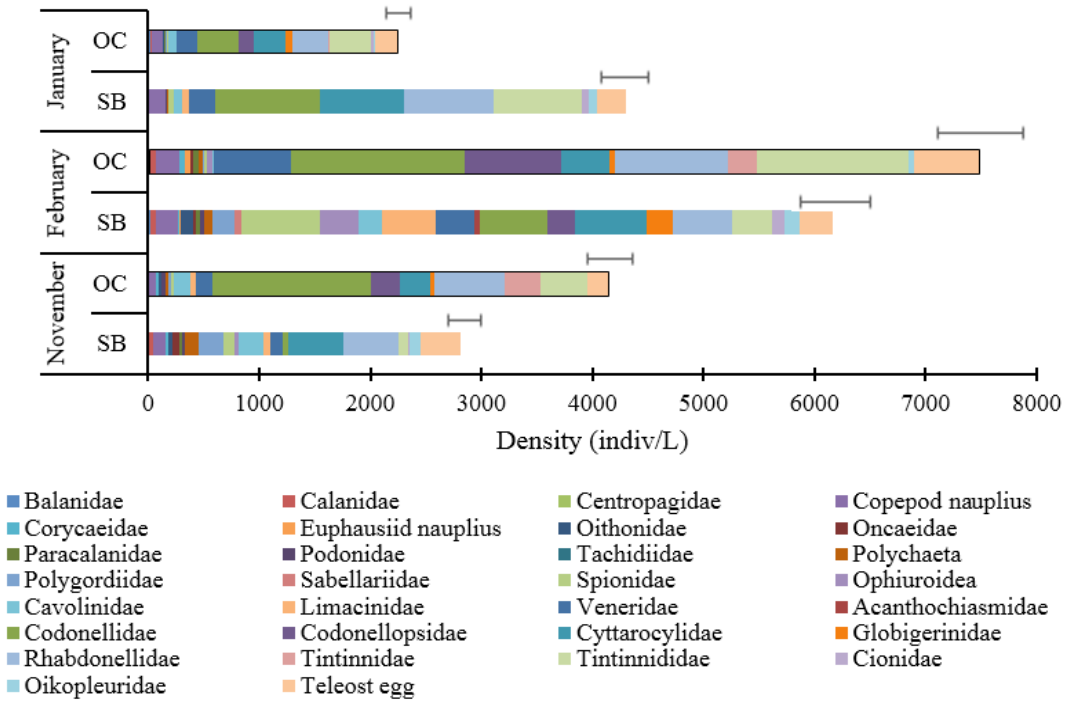

Figure 5. Mean zooplankton composition in Sogod Bay (SB) and Oslob, Cebu (OC) from November 2014 to February 2015

DISCUSSION

Physico-chemical Parameters

Whale sharks are typically found in tropical and warm temperate areas with surface temperatures of 21-25 °C (Compagno, 1984); however, they are also known to aggregate in areas with extreme temperatures of >35 °C (Robinson et al., 2013). This explains whale shark spottings in areas with surface temperatures slightly higher than the optimum such as SB (26.75 °C) and OC (27.18 °C). Nevertheless, timing of collection and influence of weather should also be considered (Jayaraman *et al*., 2003). The same trend was observed with respect to the salinity at each site, wherein SB- with a higher salinity- resulted to higher zooplankton diversity (Gonçalves *et al*., 2010). In contrast, copepod abundance was inversely proportional to pH, which follows the idea of Dar *et al*. (2009).

Water current enhances the primary productivity by transporting nutrients back to the euphotic layer. The movement of nutrients to the upper surface was a key factor to the variation of phytoplankton composition in both sites, which also affected the zooplankton composition. This follows the idea that zooplankton are also affected by the sudden change in nutrient concentrations even though not directly. Moreover, high values of nutrients may also have consequences but phosphate (0.09 µM and 0.08 µM) and nitrate concentrations (3.03 µM and 2.14 µM) were within the normal range in SB and OC, respectively. Based on the results, low currents caused waters to become more turbid (Uncles *et al*., 1992), which affects the zooplankton distribution (Hart, 1990) and predator-prey interaction (Salonen *et al*., 2009).

Zooplankton Composition, Abundance, and Diversity

Tintinnids, was the most abundant group in both sites throughout the sampling period in the present study (51.35 % in SB and 74.52 % in OC). Family Cyttarocylidae, with an average of 630 indiv/ m^3 throughout the sampling period, got the highest density in SB and Family Codonellidae, with an average of 1,100 indiv/m^3, in OC. Both families are from Order Tintinnida, which have the ability to dominate the microzooplankton community (Fonda-Umani and Beran, 2003) even though they represent only 5–10 % of the total ciliate population (Dolan, 2000). However, tintinnids do not contribute significantly to whale sharks' carbon requirement since tintinnids possess low carbon biomass (Bojanić et al., 2005). Nevertheless, together with other microzooplankton, tintinnids have been an important link between primary producers and larger organisms such as larval fish and copepods (Stoecker and Capuzzo, 1990; Telesh *et al*., 2009; Conway, 2012). In fact, ciliates are considered to be more important grazers of primary production than copepods in the Skagerrak, Denmark (Maar, 2003). In the present study, due to the sites' less warm temperature, the population of the copepods comprised only 6.65 % in SB and 4.74 % in OC, which may be a factor in the increase of tintinnid's density.

Based on our results, most zooplankton families came from Subclass Copepoda. One significance of these organisms is that they help in recycling all biogenic components in the water (Lee et al., 2009). Copepods, often termed as "insects of the seas", have the toughest exoskeleton and appendages which help them swim faster than other zooplankton (Ferdous and Muktadir, 2009). They also exhibit a variety of reproductive strategies such as high growth rates (Turner, 2004), high reproduction at warmer temperatures (Dar et al., 2009), minimal movements, low respiration rates (Marshall and Orr, 1966), and effective predator escaping and prey-capturing capabilities (Kiorboe, 2011). These characteristics enable copepods to surpass predation and become successful. Nevertheless, this group is known to be the zooplankton that comprises the diet of whale sharks (Compagno, 1984; Lavaniegos *et al*., 2012).

From 33 zooplankton groups found, Families Aegisthedae, Centropagidae, Luciferidae, and Sagittidae were exclusive in OC. The distribution of these organisms is likely dependent on salinity, temperature, and available preys (Huntsman, 1919; Bieri, 1959; Kotori, 1973; Xu, 2010; Rohner *et al*., 2015). A study conducted by Xu (2010) in the East China Sea determined that the optimal temperature and optimal salinity of different species under Family Luciferidae ranged between 26.4-28 °C and 33.2-33.8 ppt, respectively. This agrees with the results of the present study since Family Luciferidae was found in station 3 only, with an average temperature of 27.7 °C and an average salinity of 34 ppt, which is slightly higher than the optimum salinity. Moreover, sergestid shrimp *Lucifer hanseni* was the main prey of whale sharks off Mafia Island (Rohner et al., 2015). Distribution of Family Sagittidae also differs depending on their developmental stages and species (Bieri, 1959). This family constitutes 10% of the macrozooplankton biomass in Bering Sea (Kotori, 1973). Importance of other families exclusively found in OC are already discussed in the previous paragraphs.

A higher zooplankton count was observed in OC than in SB. In contrast, phytoplankton analysis revealed that higher density count was calculated in SB (33,000 indiv/m^3) compared with OC (16,000

indiv/m^3) (Gayas, 2015). The relationship observed in the present study between zooplankton and phytoplankton agrees with the study of Abdel Aziz *et al*. (2006) that both groups usually have an inverse relationship, which may be because of animal exclusion and/or grazing (Harvey *et al*., 1935; Hardy, 1936). In fact, microzooplankton, which includes small metazoans and ciliates, are the main predators of a variety of phytoplankton species (Campbell, 1926 and 1927; Gold, 1966; Beers and Stewart, 1967). Higher counts of phytoplankton can be attributed to their fast growth rate (Abdel Aziz et al., 2006) upon nutrient enrichment (Landry *et al*., 2000). Other biological and environmental factors such as temperature (Banu, 2012), water current, and composition of zooplankton and phytoplankton (Abdel Aziz *et al*., 2006; Chen *et al*., 2013) should also be considered in studying the complex nature of plankton's relationship.

Effect of Weather Disturbances

The highest density determination recorded in SB and OC were both obtained during the second sampling (January), with 7,000 indiv/m^3 in SB and 6,000 indiv/m^3 in OC. January was preceded by two weather disturbances- typhoons Ruby and Seniang, respectively. According to Merritt-Takeuchi and Chiao (2013), during weather disturbances, an internal friction is created due to strong winds. This results to the displacement of nutrients from the bottom to the water surface, allowing an efficient mixing of materials and nutrients in the water to occur. Growth of biological substances such as chlorophyll and phytoplankton attracts the zooplankton, which results to January possessing the highest density. The same trend was also observed on the phytoplankton study conducted by Gayas (2015) on both sites. Plankton blooms after a weather disturbance were also reflected on the studies of Livingston (2007), Shi and Wang (2007), Wang and Zhao (2008), Lopez-Lopez *et al*. (2012), and Merritt-Takeuchi and Chiao (2013). However, increased plankton counts after a weather disturbance are only applicable after short time intervals (Lopez-Lopez *et al*., 2012). In the study of Licayan (2014) in inner San Pedro Bay, zooplankton count increased a month after super typhoon Haiyan (2,500 indiv/m^3 in December 2013) compared to the month before the typhoon (1,800 indiv/m^3 in October 2013). The super typhoon must have stirred nutrients from the bottom, increasing phytoplankton and subsequently zooplankton abundance.

CONCLUSION

Physico-chemical parameters including pH, temperature, salinity, water current, turbidity, phosphate, nitrate analyses- obtained in Sogod Bay (SB) and Oslob, Cebu (OC) were at normal ranges which varied between months but these are generally at: 9.08 and 9.32; 26.75 ^oC and 27.18 ^oC; 34.44 ppt and 33.82 ppt; 0.06 ms^{-1} and 0.03 ms^{-1}; 0.10 NTU and 0.14 NTU, 0.09 µM and 0.08 µM, 3.03 µM and 2.14 µM, respectively.

Twenty-eight groups were found in both sites and four families (Aegisthedae, Centropagidae, Luciferidae, and Sagittidae) were exclusive in OC. Tintinnida from Class Ciliata was the most abundant order with a density of 2,000 indiv/m^3 in SB and 3,000 indiv/m^3 in OC, respectively. The order of zooplankton abundance in SB are: Ciliates (51.35%) > Polychates (32.21%) > Gastropods (8.35%) > Crustaceans (7.60%) > Others, and in OC: Ciliates (74.52%) > Bivalves (7.45%) > Teleost eggs (7.03%) > Crustaceans (5.53%) > Others.

In total, OC had a higher density value of 5,000 indiv/m^3 compared with 4,000 indiv/m^3 in SB but this is not significantly different when subjected to T-test. In contrast, SB got a higher diversity value of H'=2.75 compared with OC, which had H'=2.36. Other zooplankton observed were from classes Appendicularia, Ascidia, Bivalvia, Crustacea, Gastropoda, Ophiuroidae, Polychaeta, and Sarcodina.

The density and diversity values of mostly microzooplankton were generally high, suggesting a rich and diverse environment. However, no whale sharks were in the vicinity during the samplings in Pintuyan, Sogod Bay. Thus our results don't suggest the presence or absence of whale sharks. Further work would focus on dense prey patches that whale sharks are known to target to understand the relationship between this baseline study and detailed foraging ecology results of further work.

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